20.1 DISSECTING THE FUNCTIONAL CONSEQUENCES OF RECIPROCAL GENOMIC DISORDERS

that we unravel how these risk factors interact within and between the diverse cell types populating the brain. While mouse models are uniquely suited for demonstrating how aberrant function of single gene products contribute to aberrant neuronal function or behavior, genetic studies of penetrance and complex gene interactions are nearly impossible to address using inbred mouse lines. Similarly, the lack of human post-mortem tissue, coupled with the inability to conduct functional experiments in patient cells, has to date left us with a very limited understanding of how rare and common variants impact gene expression or cellular function. Our panelists have each developed human induced pluripotent stem cell (hiPSC)-based models for the study of predisposition to neuropsychiatric disease, establishing a new mechanism by which to systematically explore the impact of rare and common putative causal variants in human cells.

Given the heterogeneity of schizophrenia and the limited cohort sizes feasible with hiPSC-based cohorts, our panelists will share their successes and struggles in developing cohorts defined by shared clinical or genetic features. They will discuss both the molecular and phenotypic insights they have uncovered, in neurons and glia, from case/control and genetically-edited isogenic cohorts. Our discussant will focus on integrating these findings into consortia-led datasets generated from recent genomic and post-mortem studies of large schizophrenia cohorts. Our overall objective is to consider the role of hiPSC-based studies in dissecting the genetic origins of schizophrenia, validating causal variants identified through ongoing genetic analyses, and serving as a personalized medicine approach to screen for novel therapeutics with which to prevent or reverse disease course.

20.1 DISSECTING THE FUNCTIONAL CONSEQUENCES OF RECIPROCAL GENOMIC DISORDERS

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Background: Reciprocal genomic disorders (RGDs) represent a unique class of recurrent genomic variation that offer insight into highly dosage sensitive regions of the morbid human genome. However, the genomic architecture mediating RGDs, namely non-allelic homologous recombination (NAHR) of flanking segmental duplications, has rendered these genomic segments recalcitrant to conventional model studies. We recently developed a novel CRISPR method that leverages the homology of segmental duplications and efficiently generates large microdeletions and microduplications that mimic NAHR in humans, including ablation or duplication of one copy equivalent of the segmental duplications. Here, we explore the functional consequences of 16p11.2 RGD in iPS derived neuronal models and across mouse tissues.

Methods: We generated CRISPR-engineered 16p11.2 RGD models against an isogenic iPSC background and performed transcriptome profiling in iPSC-derived neural stem cells (NSCs) and induced neurons (iN) (n = 10 isogenic deletions, 10 duplications, 6 controls). We then integrated these data with RNAseq from 306 libraries from multiple tissues in 70 mouse models of reciprocal deletion and duplication of the syntenic 7qf3 region (cortex, striatum, cerebellum, liver, white fat, brown fat in 16 mice; and replication from cortex, striatum, cerebellum in 54 mice).

Results: In ongoing analyses, weighted-gene correlation network analysis (WGCNA) identified co-expression modules that were significantly enriched for 16p11.2 genes, evolutionarily constrained genes, genes robustly associated with autism spectrum disorder (ASD; TADA $q < 0.1$) and developmental disorders (DDD). Pathway analyses within modules discovered enrichment of genes critical to synaptic formation and neural connectivity as well as the protocadherin gene family. Network analyses specific to brain tissues within modules further identified a convergence on highly connected, or 'hub' genes, on Wnt signaling, including Ctnnb1 and Ctnnd1. The module was also again enriched for ASD loci (TADA, FDR < 0.1), constrained genes (ExAC, pLI ≥ 0.9) and brain specific genes from the Human Protein Atlas.

Discussion: These studies suggest the functional consequences of 16p11.2 RGD across models converge on transcriptional signatures associated with critical neurodevelopmental pathways and individual genes implicated in a spectrum of developmental and neuropsychiatric disorders.

20.2 ANALYZING THE MOLECULAR EFFECTS OF LARGE NEUROPSYCHIATRIC CNVS WITH IPSC BASED NEURONAL TISSUE CULTURE MODELS

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Background: Several large copy number variants (CNVs) in the genomic sequence are strongly associated with schizophrenia. These loci are important objects of study in their own right as well as enticing points of entry for the better understanding of the molecular etiology of schizophrenia. However, most of the schizophrenia-associated large CNVs are larger than one million base pairs and affect up to several dozen genes, presenting a complex challenge for research aiming to determine how these sequence variants are connected on the molecular level to the phenotype.

Methods: We have established iPSC based tissue culture models for three of the major schizophrenia associated large CNVs, on chromosomes 22q11 (deletion), 15q13 (deletion) and 16p11 (deletion or duplication). We create neuronal cells with the defined genotypes using either direct induction into the neuronal state (induced neurons, iNs), by slower differentiation via neuronal precursor cells (NPCs) or by generating 3D cultures of cortical spheroids. We then assay the molecular effects of the large CNVs along the trajectory of differentiation by using RNA-Seq (transcriptome), ATAC-Seq (chromatin state) and SeqCap-Epi (DNA-methylation patterns). We also carry out single-cell RNA-Seq analysis using the drop-Seq approach.

Results: We detect common effects across the large CNVs as well as locus-specific phenomena. For the most part genes within the CNV boundaries will change their expression patterns in concordance with their new copy number, with notable exceptions. Transcriptome-wide there is a network effect where several hundred genes are differentially expressed, including genes already identified as candidate genes for schizophrenia. Epigenomic states are affected, again most often not only in or nearby the boundaries of the large CNVs but epigenome-wide. Integrative analysis across the layers of molecular signals shows partial concordance as well as a degree of changes in signal being 'offset' between the levels, potentially owing to the dynamic differentiation state of the model system.

Discussion: Neuronal tissue culture models based on iPSCs with defined large CNVs strongly associated with Schizophrenia allow for an analysis of the effects of such structural genomic sequence changes in disease-relevant cellular differentiation states. Application of cutting edge genomics and epigenomics assays and integrative data analysis reveals incomplete transcriptional dosage compensation of the genes within the large CNVs as well as transcriptome-wide network effects. Furthermore, there are epigenomic effects in the form of altered chromatin states that may to some extent mediate the gene expression changes. Differences between the large CNV loci as well as potential points of convergence will be discussed.